Diagnostic Approach to Common Anemias in Pediatrics

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INTRODUCTION

Anemia is generally defined as an abnormal decrease in the number of circulating erythrocytes or in the hemoglobin concentration and the hematocrit to levels that are 2 standard deviations below the mean for the normal population. Anemia is not a diagnosis in itself; rather, it is a symptom of an underlying disease. Therefore, the evaluation of anemia is directed at elucidating the underlying cause. Table 1 and Table 2 show important features in patient history and physical findings that can yield valuable information, considerably narrowing possible causes for anemia and reducing the necessity of performing expensive tests.

COMPLETE BLOOD COUNT

The complete blood count (CBC) remains a practical starting point in the laboratory evaluation and classification of anemias and consists of hemoglobin level, hematocrit, erythrocyte count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leukocyte count, and platelet count. The CBC results determine whether the problem is anemia alone, anemia with leukopenia or thrombocytopenia, or pancytopenia, entities that would significantly alter the differential diagnosis. On newer cell counters, the red cell distribution width (RDW) is available and serves as a quantitative measure of the degree of variability in the size and shape of the erythrocytes (anisocytosis—excessive variation in size; poikilocytosis—excessive variation in shape). The reticulocyte count, a measure of bone marrow erythrocyte production, is another helpful parameter in the initial work-up. Lastly, examination of the peripheral blood smear can be particularly useful in confirming the results of the CBC, as well as providing morphologic clues to the diagnosis.

When analyzing the CBC, it is important to consider developmental variations in reference to the hemoglobin level, the hematocrit, and erythrocyte indices in the infant or child. Normal values in the pediatric years are listed in Table 3. Hemoglobin levels and hematocrit are relatively high in the newborn period, with mean values of 18.5 g/dL and 56%, respectively. At birth, the infant moves from a relatively hypoxic intrauterine environment and an arterial oxygen saturation of 45% to a normoxic environment where arterial saturation increases to 95% with the onset of respiration. This change causes an abrupt cessation in erythropoietin production and a halt in erythropoiesis. In addition, fetal erythrocytes in the newborn have a shortened lifespan of 60 to 70 days, compared to the 120-day lifespan of adult erythrocytes. The rapid growth of the newborn baby is accompanied by an increase in plasma volume, which results in a dilutional effect to further decrease hemoglobin levels and hematocrit. A nadir is reached at approximately 6 to 8 weeks of age for the term infant and earlier—at around 5 weeks of age—for the premature infant. Hemoglobin and hematocrit levels then steadily increase, reaching adult levels at puberty. This condition is referred to as physiologic anemia of infancy and is an extraterine adaptation to life rather than a pathologic anemia.

CLASSIFICATION OF ANEMIAS

Anemias may be classified on the basis of erythrocyte size (MCV). Microcytic, hypochromic anemias are the most common class of anemias in pediatric years. The MCV is below the lower limit of normal for age. In children between the ages of 2 years to 10 years, the lower limit of normal for MCV is 70 fL plus the age in years. These anemias are caused by impaired synthesis of the heme or globin components of hemoglobin.

Normochromic, normocytic anemias are characterized by an MCV in the normal range. They are further classified by the reticulocyte count and are caused by hemorrhage, hemolysis, or hypoproduction of the bone marrow.

Macrocytic anemias are characterized by an MCV above the upper limit of normal, which is obtained by adding 0.6 fL per year to 84 fL beyond the first year of life until the upper limit of 96 fL in adults is reached.
These anemias also are further classified by the reticulocyte count (indicating hemolysis or bone marrow failure), and by the presence or absence of megaloblastic changes (indicating nutritional deficiencies).

This review focuses on the most common causes of anemia in children within the context of the changing epidemiologic pattern of childhood anemia. Emphasis is placed on microcytic, hypochromic anemias, as this is the most common group seen in pediatrics. A brief overview of normochromic, normocytic anemias, as well as of macrocytic anemias, which are even less common, is provided in order to review their diagnostic approach.

### Microcytic, Hypochromic Anemias

#### Case 1 Presentation

Patient 1 is a 12-month-old African American boy who presents for a 1-year well child check. His mother reports that he has been doing well except for 2 episodes of otitis media in the past 2 months. He completed a 10-day course of amoxicillin/clavulanate potassium 2 weeks ago, and has been doing well since that time. He is on an iron-fortified formula and eats a balanced diet of table food with some baby food. Results of the physical examination are normal. After he leaves the clinic, the CBC is reported to include a hemoglobin level of 10.0 g/dL and a MCV of 69 fL, with normal leukocyte and platelet counts.

- Should the patient have further evaluation of this mild anemia?
- Would an empiric trial of iron be appropriate?
- Should screening be done for thalassemia trait or lead toxicity?

#### Differential Diagnosis of Microcytic Anemias

In normal hematopoiesis, erythrocytes are thought to be released from the marrow when they achieve their full complement of hemoglobin. Microcytic, hypochromic anemias are associated with defects in hemoglobin production. This is because the erythrocyte keeps dividing until it reaches a reasonable hemoglobin content, at which time it is released into the peripheral blood. Defective heme synthesis may result from iron...
deficiency, abnormal iron metabolism (owing to acute or chronic inflammation or infection), metabolic abnormalities (sideroblastic anemias), or toxicity (eg, lead poisoning). Defective globin synthesis is characteristic of the thalassemia syndromes.

Iron-Deficiency Anemia

Iron deficiency is the most common cause of anemia in children. Most cases occur because the dietary intake of iron is inadequate to meet the requirements for rapid growth. This is mainly seen in infants and results

| Table 2. Physical Findings as Clues to the Etiology of Anemia in Children |
|-----------------------------|-----------------------------|
| **Finding**                 | **Possible Etiologies**     |
| **Skin**                    |                             |
| Hyperpigmentation           | Fanconi’s aplastic anemia   |
| Petechiae, purpura          | Autoimmune hemolytic anemia with thrombocytopenia, hemolytic-uremic syndrome, bone marrow aplasia, bone marrow infiltration |
| Carotenemia                 | Suspect iron deficiency in infants |
| Jaundice                    | Hemolytic anemia, hepatitis, aplastic anemia |
| cavernous hemangioma        | Microangiopathic hemolytic anemia |
| Ulcers on lower extremities | S and C hemoglobinopathies, thalassemia |
| **Eyes**                    |                             |
| Microcornea                 | Fanconi’s aplastic anemia   |
| Tortuosity of the conjunctival and retinal vessels | S and C hemoglobinopathies |
| Microaneurysms of retinal vessels | S and C hemoglobinopathies |
| Cataracts                   | G6PD deficiency, galactosemia with hemolytic anemia in newborn period |
| Vitreous hemorrhages        | S hemoglobinopathy          |
| Retinal hemorrhages         | Chronic, severe anemia      |
| Edema of the eyelids        | Infectious mononucleosis, exudative enteropathy with iron deficiency, renal failure |
| **Facies**                  |                             |
| Frontal bossing, prominence of the malar and maxillary bones | Congenital hemolytic anemias, thalassemia major, severe iron deficiency |
| **Mouth**                   |                             |
| Glossitis                   | Vitamin B₁₂ deficiency, iron deficiency |
| Angular stomatitis          | Iron deficiency             |
| **Chest**                   |                             |
| Unilateral absence of the pectoral muscles | Poland’s syndrome (increased incidence of leukemia) |
| Shield chest                | Diamond-Blackfan syndrome   |
| **Hands**                   |                             |
| Triphalangeal thumbs        | Red cell aplasia            |
| Hypoplasia of the thenar eminence | Fanconi’s aplastic anemia |
| Spoon nails                 | Iron deficiency             |
| **Spleen**                  |                             |
| Enlargement                 | Congenital hemolytic anemia, leukemia, lymphoma, acute infection, portal hypertension |

from a failure to introduce iron-containing foods at the age of 5 to 6 months (for full-term infants), the time when the endowment of iron provided in the last trimester of pregnancy has been exhausted. In premature infants, anemia may occur before 6 months of age because iron stores at birth may have been inadequate owing to curtailment of the pregnancy. Iron-deficiency anemia also occurs during adolescence, when rapid growth puts excessive demands on iron stores; this is especially a problem in girls, who lose iron with menses.

It is always important to rule out iron deficiency resulting from blood loss. Prenatal iron loss can result from fetomaternal transfusion or twin-to-twin transfusion. In older infants and children, blood loss may be occult and may be caused by parasitic infestations, inflammatory bowel disease, Meckel’s diverticulum, polyps, cow’s milk enteropathy, celiac disease, drugs (eg, nonsteroidal anti-inflammatory agents), or idiopathic pulmonary hemosiderosis.

Anemia of Inflammation

Anemia of inflammation is second in incidence only to iron-deficiency anemia. Since the advent of the Women, Infants, and Children (WIC) program in the United States, the incidence of anemia due to iron deficiency has declined and may eventually be surpassed by anemia of inflammation. The inflammation may be secondary to infection, collagen vascular disease, or malignancy. The association between various infections and anemia has long been observed, but it has only been in recent years that the role of mild and common childhood infections (eg, otitis media, upper respiratory tract infection, gastroenteritis) in causing anemia has been clarified. Fever of longer than 3 days’ duration or recent immunization with live measles virus vaccine also can mildly depress the hemoglobin level, causing anemia.

Sideroblastic Anemias

Sideroblastic anemias are rare in children and occur because of a metabolic dysfunction in which the incorporation of iron into hemoglobin is blocked. Instead, iron accumulates in the mitochondria found in a perinuclear distribution and imparts a characteristic ring around the nucleus when stained for iron content. Therefore, sideroblastic anemias are identified by an excess of ringed sideroblasts in the marrow (>10% of nucleated cells) having 4 or more siderotic, perinuclear granules (representing iron-laden mitochondria). Conditions that result in sideroblastic anemia in childhood are pyridoxine deficiency, ptyaline deficiency, and lead poisoning.

Plumbism (lead poisoning) may be combined with, and enhanced by, iron deficiency in children with pica. Some of the effects of plumbism may be attributed to inhibition of ferrochelatase, the enzyme that incorporates iron into the protoporphyrin ring, resulting in increased levels of free erythrocyte porphyrin. Inhibition

<table>
<thead>
<tr>
<th>Table 3. Normal Erythrocyte Values in the Pediatric Years</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>1–3 days (term infant)</td>
</tr>
<tr>
<td>1 month</td>
</tr>
<tr>
<td>2 months</td>
</tr>
<tr>
<td>3–6 months</td>
</tr>
<tr>
<td>6 months–2 years</td>
</tr>
<tr>
<td>2–6 years</td>
</tr>
<tr>
<td>6–12 years</td>
</tr>
<tr>
<td>12–18 years</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Male</td>
</tr>
</tbody>
</table>

MCV = mean corpuscular volume.

*Mean and lower limit of normal. Lower limit is 2 standard deviations below the mean.

of pyrimidine-5′-nucleotidase, the enzyme responsible for cleaving the nucleotides that remain after nuclear extrusion from the erythrocyte, accounts for the ribosomal RNA and mitochondrial fragments that appear as coarse basophilic stippling in cells affected by plumbism.7

**Thalassemias**

The thalassemias are hereditary defects in the synthesis of normal globin polypeptide chains. Patients with anemia caused by mild forms of thalassemia are encountered frequently in clinical practice. However, the severe forms are relatively rare and are blatant hemolytic anemias that are not easily confused with other hypochromic, microcytic anemias.

The overall prevalence of the α-thalassemia trait is approximately 3% to 8% in Americans of Greek or Italian ancestry, 5% to 15% in those of southeast Asian ancestry, and 6% to 11% in African Americans. In addition, more than 20% of southeast Asian immigrants and up to 24% of African Americans are estimated to be silent carriers. The β-thalassemia trait occurs with increased frequency in African Americans (1%) and Southeast Asians (4%).5,8

**LABORATORY EVALUATION**

In patients with a microcytic anemia, details of the history and physical examination should always guide selection of tests to arrive at a definitive diagnosis in a cost-effective manner. Characteristic features of the CBC and peripheral blood smear also should be noted, and are presented in Table 4. Anemia of inflammation is similar to iron deficiency anemia; both are characterized by hypochromia and microcytosis. Anisocytosis and poikilocytosis (as manifested by a high RDW) are characteristic of iron deficiency but are not seen in the anemia of inflammation. In early or mild iron deficiency, however, these morphologic abnormalities may be absent.

The hypochromia and microcytosis of thalassemia are generally of a greater magnitude than that observed in the same degree of anemia in the anemia of inflammation or in iron deficiency. MCV and MCHC are therefore lower in patients with thalassemia. In addition, elevated erythrocyte counts and the presence of target cells and cells exhibiting basophilic stippling are commonly seen in patients with thalassemia but not in those with anemia of inflammation or iron deficiency. The Mentzer index (erythrocyte count divided by the MCV) is useful in distinguishing thalassemia minor from iron deficiency. A Mentzer index of less than 13.5 suggests thalassemia minor, whereas an index of greater than 13.5 suggests iron deficiency.

Hematologic features useful in distinguishing lead poisoning from iron deficiency include basophilic stippling,

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**Table 4. Pertinent Findings in Microcytic Hypochromic Anemia**

<table>
<thead>
<tr>
<th>Cause of Anemia</th>
<th>Erythrocyte Count</th>
<th>Red Cell Distribution Width</th>
<th>Anisopoikilocytes</th>
<th>Basophilic Stippling</th>
<th>Bone Marrow Iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency</td>
<td>Decreased</td>
<td>Normal or increased</td>
<td>Yes</td>
<td>No</td>
<td>Decreased</td>
</tr>
<tr>
<td>Thalassemia minor</td>
<td>Normal or increased</td>
<td>Normal</td>
<td>No</td>
<td>Yes</td>
<td>Increased</td>
</tr>
<tr>
<td>Sideroblastic anemias</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hereditary</td>
<td>Decreased</td>
<td>Variable</td>
<td>Variable</td>
<td>Yes</td>
<td>Increased ringed sideroblasts</td>
</tr>
<tr>
<td>Acquired</td>
<td>Decreased</td>
<td>Dimorphic population</td>
<td>Yes</td>
<td>Yes</td>
<td>Increased ringed sideroblasts</td>
</tr>
<tr>
<td>Chronic disease</td>
<td>Decreased</td>
<td>Variable</td>
<td>Variable</td>
<td>No</td>
<td>Decreased in siderocytes; increased in RE cells</td>
</tr>
</tbody>
</table>

RE = reticuloendothelial.

poor response to iron treatment, and ringed sideroblasts in the marrow. Of note, the basophilic stippling in lead poisoning is coarser than that seen in thalassemia. In addition, sideroblastic anemias have a characteristic dimorphic population of microcytic and normocytic or macrocytic cells.

Because iron deficiency is the most common cause of anemia and has adverse effects on behavior and development, iron studies serve as a useful guide in the initial work-up of hypochromic, microcytic anemias. Tests to evaluate iron status include serum iron level, total iron-binding capacity (TIBC), serum ferritin level, and serum soluble transferrin receptor level. It is important to note that serum iron concentrations show wide diurnal variations, with highest levels obtained in the morning. Therefore, samples should be obtained in the morning. Although bone marrow examination is usually not clinically indicated to confirm the diagnosis of iron-deficiency anemia, it is the most direct means for assessing body iron stores. Table 5 shows results of iron studies typical of various hypochromic anemias. A trial of iron supplementation is commonly used to diagnose iron deficiency instead of obtaining iron studies when the history, physical examination, and CBC with differential are suggestive. A 1-month trial of oral iron at a dosage of 6 mg of elemental iron/kg of body weight divided into 2 or 3 doses daily should result in a more than 1 g/dL increment increase in hemoglobin.

If thalassemia is suspected on the basis of a high erythrocyte count and a Mentzer index of less than 11.5, hemoglobin evaluation should be performed using electrophoresis, high performance liquid chromatography (HPLC), or isoelectric focusing. In patients with β-thalassemia trait, hemoglobin evaluation usually reveals an increase in hemoglobin A₂ and hemoglobin F, whereas in patients with α-thalassemia trait, findings may be normal unless performed in the newborn period. Family studies are useful in the evaluation of patients with thalassemia trait.

Other studies that may be performed in the evaluation of patients with microcytic, hypochromic anemias include the erythrocyte sedimentation rate, which is usually elevated in infection and inflammation. Urinalysis and stool guaiac testing should be performed to rule out occult blood loss. Although bone marrow examination is not usually required for the evaluation of microcytic, hypochromic anemias, it is important in the diagnosis of sideroblastic anemia.

EVALUATION OF PATIENT 1

The remainder of the CBC results come back. The erythrocyte count is $4.1 \times 10^{12}/\text{mm}^3$ (normal, $3.8–5.2 \times 10^{12}/\text{mm}^3$), the RDW is 12 (normal, $< 14$) and the blood smear is normal. These results, along with the history of recent infection, suggest the diagnosis of anemia of inflammation. The most prudent approach is to wait for the inflammation to resolve prior to further evaluation. A repeat CBC is performed 1 month later with normal results, and no further evaluation is necessary.

ANEMIA OF INFLAMMATION

Pathophysiology

Serum iron levels and TIBC are labile. After the onset of acute inflammation, both the serum iron and the TIBC fall within the first 24 hours and decrease to almost half of their previous levels within 48 hours. The macrophages that store iron bind to the iron excessively, preventing its release to the marrow for erythropoiesis. In the presence of infection, this lability probably protects
the host by partially inhibiting bacterial growth and subsequent invasion by decreasing the amount of iron available to the organisms. Serum iron levels are low not only because macrophages do not release iron, but also because of decreased absorption from the gut. These changes may persist for a month or more as the inflammation resolves. Therefore, children should be screened for anemia when they are well.

Clinical Presentation

Anemia of inflammation is a benign, mild to moderate anemia. Antecedent infections as well as immunizations can significantly affect hemoglobin concentration resulting in anemia. In addition, collagen vascular diseases and malignancy can play a role in the development of anemia. The anemia resolves when the underlying disease process is adequately treated. Therefore, a diligent search to determine the exact nature of the inflammation is essential.

Management of Anemia of Inflammation

If anemia of inflammation is considered likely, the best approach is to wait for resolution of the inflammation before pursuing a work-up. Resolution may take 1 to 3 months. In the meantime, treatment should be directed at investigating the underlying cause of inflammation, if not already known. Iron therapy is not indicated and should be avoided. Blood transfusion should be considered only if the patient is symptomatic from the anemia, a rare occurrence when the hemoglobin level is above 7 g/dL, as in the case patient. One interesting approach to treatment of patients with severe anemia of inflammation (hemoglobin 7–8 g/dL or less) is the use of recombinant human erythropoietin, which has shown some promise in adult studies, but awaits further investigation in pediatric patients.10–12

NORMOCHROMIC, NORMOCYTIC ANEMIAS

CASE 2 PRESENTATION

Patient 2 is an 18-month-old African American girl who was brought to the emergency room with low-grade fever, decreased appetite, and painful swelling of her hands. Her parents report that they gave her a dose of acetaminophen at home, but she did not show any signs of relief. On physical examination, she is fussy but consolable and is tachycardic with a grade III/VI systolic ejection murmur. The spleen is palpable 3 cm below the left costal margin. The CBC reveals a hemoglobin level of 8.3 gm/dL, a hematocrit of 24%, MCV of 78 fL, erythrocyte count of $3.2 \times 10^{12}/\text{mm}^3$, and a reticulocyte count of 8%. The peripheral blood smear shows some target cells and a rare sickle cell.

- What test would establish this patient’s definitive diagnosis?

DIFFERENTIAL DIAGNOSIS OF NORMOCHROMIC, NORMOCYTIC ANEMIAS

Normochromic, normocytic anemias are a heterogeneous group of disorders that can be further classified by the reticulocyte count. The normal range of the reticulocyte count in the absence of anemia is shown in Table 3. Normochromic, normocytic anemias are caused by hemorrhage, hemolysis, or hypoproduction of erythrocytes by the bone marrow. Elevated reticulocyte counts are indicative of hemorrhage or hemolysis. A low reticulocyte count usually suggests bone marrow failure.

Reticulocytosis

Reticulocytosis in the presence of a normochromic, normocytic anemia and without evidence of blood loss is caused by a hemolytic process. Differential diagnosis of hemolytic anemias rests largely in the recognition of specific morphologic abnormalities on the peripheral blood smear followed by appropriate specific laboratory tests.

Hemolytic anemias are caused either by inherited intracorpuscular defects or by acquired extracorpuscular defects. Intracorpuscular defects involve abnormalities of the erythrocyte membrane, enzymes, or globin. Membrane defects include hereditary spherocytosis, elliptocytosis, or stomatocytosis. Paroxysmal nocturnal hemoglobinuria is an uncommon, nonhereditary membrane defect in which erythrocytes acquire an abnormal sensitivity to complement. Enzymopathies generally involve either the glycolytic pathway (the Embden-Meyerhof pathway) or the hexose monophosphate shunt. The most common glycolytic enzyme deficiency is that of pyruvate kinase, whereas the most common hexose monophosphate shunt enzyme deficiency is that of glucose-6-phosphate dehydrogenase (G6PD). Hemoglobinopathies (eg, sickle cell diseases, thalassemias) result from a qualitative or quantitative change in one of the globin polypeptide chains.

Antibody-mediated hemolytic anemias can be autoimmune or isoimmune. Autoimmune hemolytic anemias are caused by antibodies generated within an individual against self erythrocytes. These anemias may be idiopathic or may result from infection, drugs, malignancy, or collagen vascular diseases. Isoimmune
hemolytic anemias result from antibodies produced by one individual against erythrocytes from another individual. These include hemolytic disease of the newborn and hemolytic transfusion reactions.

Microangiopathic hemolytic anemias result from mechanical damage to the erythrocytes caused by irregularities in the vascular endothelium. These occur in association with disseminated intravascular coagulation and hemolytic-uremic syndrome.

**Reticulocytopenia**

Normochromic, normocytic anemia with reticulocytopenia usually suggests bone marrow failure resulting in pure red cell aplasia or pancytopenia. Pure red cell aplasias can be congenital or acquired. The congenital form, transmitted in an autosomal fashion, is Diamond-Blackfan anemia. Although considered here with normocytic anemias, Diamond-Blackfan anemia is usually macrocytic. Acquired pure red cell aplasia, or transient erythroblastopenia of childhood, most likely results from suppression by an antecedent viral infection, although a specific cause has not been well documented. Viral infection with parvovirus-B19 can lead to aplastic crisis in patients with a chronic hemolytic disorder as well as in immunocompromised patients who are unable to mount an antibody response and clear the infection.

**EVALUATION OF PATIENT 2**

A history of dactylitis in the hands and feet of an African American child should raise high suspicion for sickle cell disease (SCD). This patient’s physical findings are characteristic of the hand-foot syndrome caused by vaso-occlusive crisis. Splenomegaly is often seen in children with SCD, but the spleen has auto-infarcted by age 5 years in the severe forms. Hemoglobinopathies often result in a characteristic peripheral blood smear which is also diagnostic. The presence of sickle cells on the smear does establish the diagnosis of some form of SCD in this patient.

**SICKLE CELL DISEASE**

**Pathophysiology**

SCD is a group of hemoglobinopathies estimated to affect more than 70,000 African Americans. It is the most common cause of hemolytic anemia in the African American population, and the gene prevalence among African Americans is 8%. More than 2000 babies with SCD are born in the United States each year, making it the most prevalent genetic disease among African Americans. However, this represents only a small percentage of the total cases worldwide. It has been estimated that in Africa alone, 120,000 babies are born each year with SCD. SCD is characterized by the production of sickle hemoglobin (Hb S) due to an abnormal gene that substitutes valine for glutamic acid in the sixth position of the β-globin chain. Homozygous individuals have sickle cell anemia (Hb SS), whereas compound heterozygous individuals have sickle hemoglobin C disease (Hb SC), sickle beta zero (Sβ0) thalassemia, or sickle beta plus (Sβ+) thalassemia. These 4 genotypes account for most cases of SCD in the United States. Generally, individuals with Hb SS and Sβ0 thalassemia are more severely affected than children with Hb SC or Sβ+ thalassemia.

The pathogenesis of SCD has been attributed to polymerization of deoxygenated Hb S, leading to altered shape and deformability of the erythrocyte. The clinical consequences are 2-fold, manifesting as chronic hemolytic anemia and vaso-occlusion. The many complications encountered in SCD include recurrent pain episodes, infection, splenic and liver dysfunction, and central nervous system involvement. The clinical course (and therefore, treatment) of SCD varies and is severe in Hb SS, moderate to severe in Sβ0 thalassemia and mild to moderate in Sβ+ thalassemia and Hb SC.

Fetal hemoglobin (Hb F) is present in large amounts at birth and has been shown to inhibit sickling in vitro by interfering with the polymerization of Hb S. As levels of Hb F decrease, the level of Hb S increases. Because of the protective effects of Hb F, the clinical manifestations of SCD generally do not occur until the infant is a few months old.

The earliest clinical manifestation of SCD in many infants occurs at approximately 6 months of age, and is characterized by painful swelling of the dorsum of the hands and feet. This hand-foot syndrome is caused by vaso-occlusion in the metacarpal and metatarsal bones, resulting in ischemic tissue injury. During this same period, progressive anemia with jaundice and splenomegaly develops and is variable among different forms of SCD. Tissue damage to the spleen can lead to functional asplenia, making overwhelming infection with encapsulated organisms (primarily pneumococcus), the most common cause of death. As with other manifestations of SCD, the development of splenic atrophy and dysfunction varies among the different forms of SCD.

In a national multicenter study, penicillin prophylaxis resulted in an 84% decrease in the incidence of pneumococcal bacteremia in young children with sickle cell anemia and has become the standard of care for children with Hb SS and Sβ0 thalassemia. Routine use of penicillin prophylaxis for infants and children with Hb SC and Sβ+ thalassemia is controversial because the increased risk of severe infection is less than in Hb SS and Sβ0 thalassemia. Recognizing that early detection and
institution of penicillin prophylaxis can prevent death and serious morbidity in infants who have sickle cell anemia. 46 states in this country have instituted mandatory newborn screening for SCD. The other 4 states have instituted screening for selected populations, universal or limited pilot programs, or screening by request.17

Another leading cause of death in children with SCD is the acute splenic sequestration crisis. In Hb SS and S\(\beta^+\) thalassemia, these episodes can occur as early as 2 months of age and are unusual after 3 years of age. In fact, splenomegaly may persist in other forms of disease (Hb SC and S\(\beta^+\) thalassemia) and splenic sequestration crises can occur in older children and adults. Most of these episodes are mild, but severe and fatal episodes have occurred in some patients.

For reasons that are not clearly understood, the spleen suddenly traps a large portion of the blood volume. Patients may suddenly become weak, tachycardic, and dyspneic; and have a rapidly distending abdomen, left-sided abdominal pain, and shock. Sequestration may also take place in the liver, but because it is not as distensible as the spleen, there is seldom pooling of a significant amount of blood to cause cardiovascular collapse.

Vaso-occlusion and tissue ischemia in SCD can result in acute or chronic injury to virtually every organ of the body. However, a detailed discussion of the diverse clinical manifestations of SCD is beyond the scope of this review.

**Laboratory Evaluation of Hemoglobinopathies**

Hemoglobin electrophoresis is the principal procedure used to separate, detect, and identify abnormal hemoglobins. In some laboratories, this is supplemented or replaced by HPLC analysis. Normal adult hemoglobin is a tetrameric protein composed of 4 globin polypeptide chains: 2 alpha and 2 beta chains that pair (designated Hb A). Although adult hemoglobin is present at birth, Hb F (Hb \(\alpha_2\gamma_2\)) predominates at birth and during the first few months of life. Within the first year of life, however, Hb F is replaced by the other adult hemoglobins. The normal adult proportion of hemoglobins is 97% Hb A (Hb \(\alpha_2\beta_2\)), 2% Hb A\(_2\) (Hb \(\alpha_2\delta_2\)) and 1% Hb F. Higher levels of Hb F are associated with less severe disease, and knowing the Hb F level can therefore help with prognosis. Repeat tests at an older age may be required to establish the stable Hb F level. Hemoglobin evaluation studies also should be performed on family members, if possible.

**Management of Sickle Cell Disease**

Patient 2 should be hospitalized for further management. Therapy for pain control includes administration of intravenous fluids and parenteral opioids. In addition, blood and other appropriate cultures should be obtained, and broad-spectrum antibiotics should be started as soon as possible. The spleen size should be monitored frequently for any signs of acute sequestration crisis.

At discharge, prophylactic penicillin should be started at a dose of 125 mg twice daily and continued until the child is 5 years old. Ongoing comprehensive medical care should be provided, including extensive parental education, timely immunizations, prompt evaluation and aggressive management of complications, genetic counseling, and hemoglobin evaluation studies also should be performed on family members.

Hydroxyurea therapy has been shown to ameliorate the clinical course of sickle cell anemia in adults by increasing the production of Hb F and inhibiting sickling.16–18 Concerns about potential adverse effects on growth as well as the possibility of teratogenic or carcinogenic effects led to an initial reluctance to use hydroxyurea in children. Phase I and II multicenter pediatric trials using hydroxyurea in children ages 5 to 16 years with severe sickle cell anemia have shown no adverse effects on height, weight gain, or pubertal development.19 Recently, the results of a 2-year pilot study in very young children (ages 6 to 24 months) showed that hydroxyurea may prove to be effective not only in preventing pain, but also in delaying or preventing the organ damage associated with SCD.20

Successful allogeneic bone marrow transplantation (BMT) provides a hematologic cure for SCD. Currently, the event-free survival rate following BMT for SCD is 82%.21 The short-term and long-term transplant-related complications remain substantial barriers to performing BMT to all patients with SCD who would benefit. Novel conditioning regimens that minimize transplant-associated toxicity have been developed and show promise for wider application of BMT.21,22 Perhaps in the future, gene therapy for hemoglobinopathies also will be possible.

**MACROCYTIC ANEMIAS**

**CASE 2 CONTINUATION**

Patient 2 is discharged from the hospital after 3 days, and is placed on penicillin prophylaxis. She does well for 2 years. When she is 3 years old, her hematocrit is noted to gradually fall to a level of 18% with a decrease in her reticulocyte count to 3%. The MCV is noted to increase to 96 fL. The peripheral blood smear reveals target cells and sickled cells, as were noted previously, but these cells are now accompanied by an increase in
hypersegmented neutrophils and the presence of some oval macrocytes.

- What is the most likely cause of patient 2’s macrocytic anemia?
- Is it related to her SCD?

DIFFERENTIAL DIAGNOSIS OF MACROCYTIC ANEMIAS

Macrocytic anemias are characterized by MCVs above the upper limit of normal and are not common in pediatric patients. Macrocytic anemias may be associated with megaloblastosis, indicating decreased DNA synthesis and asynchrony of maturation between cytoplasm and nucleus. This results in impaired synthesis of DNA relative to RNA and protein synthesis. Therefore, these anemias result from a relative decrease in the factors needed in RNA and protein synthesis. Other consequences include asynchrony in maturation between the cytoplasm and nucleus resulting in impaired synthesis of DNA relative to DNA replication and are usually caused by a deficiency of folate, vitamin B₁₂, or both. Except in infants, total body DNA replication and are usually caused by a deficiency of methylmalonic acid is elevated in vitamin B₁₂ deficiency because this nutrient is required for the conversion of homocysteine to methionine. Vitamin B₁₂ is required for the conversion of methylmalonyl CoA to succinyl CoA; therefore, methylmalonic acid is elevated in vitamin B₁₂ deficiency and is a helpful diagnostic aid.

Morphologic abnormalities characteristic of folate deficiency may be seen on the peripheral blood smear. Hypersegmentation of neutrophils is among the earliest findings, and neutropenia is present in severe cases. Macro-ovalocytosis and anisocytosis are typical erythrocyte abnormalities. In severe cases, thrombocytopenia is seen.

FOLATE DEFICIENCY

Pathophysiology

Folate deficiency is the most common cause of megaloblastic anemia in the general population. Folate plays an important role as a carrier of single-carbon fragments, such as methyl, formyl, and methylene groups. Of major importance in erythrocyte production is the reaction in which deoxyuridylate is converted to thymidylate. This reaction, which appears to be rate-limiting in DNA synthesis, requires methylene tetrahydrofolate.²² Folate also plays an important role in central nervous system methylation of homocysteine to methionine.

Biochemical manifestations of folate deficiency include asynchrony in maturation between the cytoplasm and nucleus resulting in impaired synthesis of DNA relative to RNA and protein synthesis. Other consequences include ineffective erythropoiesis with intramedullary hemolysis and impaired utilization of iron.

Management

The diagnosis of folate deficiency is confirmed by the demonstration of a decreased folate level and a hematologic response to a test dose of folic acid. Pediatric patients with folate deficiency should receive 0.5 to 1.0 mg of orally administered folic acid daily until the anemia and megaloblastosis are corrected. Patients diagnosed with chronic hemolytic anemias should take folic acid 1 mg daily to prevent folate deficiency.
SUMMARY

The initial diagnostic approach to the anemic patient includes a detailed history and physical examination and a minimum of essential laboratory tests. Careful scrutiny of the peripheral blood smear is always helpful and may provide morphologic clues to the diagnosis, thereby minimizing expensive and unnecessary tests.

The pattern of anemia in infancy and childhood has changed over the past decade. Although iron-deficiency anemia remains the most common cause of anemia in the United States, its incidence is declining, and the anemia of inflammation may eventually surpass it. The growing Asian and African American populations in the United States along with newborn screening for hemoglobinopathies has resulted in more frequent identification of these disorders. Furthermore, during the course of a disease, classification of a patient’s anemia may change from one category to another as a result of additional clinical or pathologic variables.

REFERENCES


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